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REMARKS

Claims 50-57 are pending in the subject application. No claim has been added, canceled or amended herein. Accordingly, claims 50-57 are still pending and under examination.

In view of the arguments set forth below, applicant maintains that the Examiner's objections and rejections made in the April 14, 2004 Final Office Action have been overcome, and respectfully requests that the Examiner reconsider and withdraw same.

Rejection Under 35 U.S.C. §103(a) - Obviousness

The Examiner rejected claims 50-57 under 35 U.S.C. §103(a) as allegedly unpatentable over Columbo (J. of Immunology) in view of Mohammadi (Science).

In response to the Examiner's rejection of claims 50-57, applicant respectfully traverses, and maintains that the Examiner has failed to establish a *prima facie* case of obviousness against the rejected claims.

Claims 50-57 provide methods for treating or preventing cutaneous inflammation. These methods comprise administering to a subject an amount of an antibody that binds to kit protein, thereby treating or preventing cutaneous inflammation.

To establish a *prima facie* case of obviousness, the Examiner

must demonstrate three things with respect to each claim. First, the cited references, when combined, must teach or suggest each element of the claim. Second, one of ordinary skill would have been motivated to combine the teachings of the cited references at the time of the invention. And third, there would have been a reasonable expectation that the claimed invention would succeed.

Here, the references cited against the rejected claims fail to support a *prima facie* case of obviousness. Specifically, to support a *prima facie* case of obviousness, one of ordinary skill would have to have been motivated to combine the teachings of the cited references at the time of the invention. Moreover, these references would also have to provide a reasonable expectation of success.

Columbo only teaches a human recombinant c-kit receptor ligand stem cell factor (rhSCF) and its effects on the release of inflammatory mediators from human skin mast cells and peripheral blood basophils *in vitro*. Nowhere is it suggested that anti c-kit receptor ligand or antibody can be used *in vivo* to treat or prevent *any* disease, let alone cutaneous inflammation, via blocking the SCF-KIT signaling pathway.

Mohammadi does nothing more than disclose a new class of protein tyrosine kinase inhibitors based on an oxindole core (indolinones) and its effect on fibroblast growth factor receptor 1 (FGFR1) in NIH 3T3 cells. Although Mohammadi states that selective inhibitors of protein tyrosine kinases have considerable therapeutic value, this statement, at most applies solely to the treatment of some forms of cancer, such

as glioma, through the use of indolinones. (Mohammadi, page 959). Nowhere is it suggested that indolinones are anti-c-kit ligands or that an anti-c-kit ligand can be used to prevent or treat *any* disease, let alone cutaneous inflammation *in vivo*.

To support a case of *prima facie* obviousness, Columbo and Mohammadi, when combined, would also have to teach or suggest all elements of the rejected claims. Moreover, again, these references would have to create a motive to combine such elements, and a reasonable expectation of the invention's success.

Applicant maintains that the combination of Columbo and Mohammadi would not have supported a reasonable expectation of success of the claimed methods by one of ordinary skill at the time of invention. Columbo and Mohammadi teach only *in vitro* experiments and data, without sharing *in vivo* data relating to the therapeutic use of inhibitors of KIT protein in relation to inflammation. For reasons detailed below, applicant stresses that one could not have predicted the success of the claimed invention based on *in vitro* experiments.

The Examiner asserts that one routinely bases *in vivo* methods upon *in vitro* experiments which is why *in vitro* experiments are performed, and one would have a high expectation of success in doing so.

Applicant respectfully disagrees. A high expectation of success cannot be equated with what is, at most, an incentive to experiment. Although *in vitro* experiments may provide an incentive to create *in vivo* models for further

experimentation, there still remains uncertainty as to whether *in vitro* data can be replicated in *in vivo* models. The complexity of an *in vivo* model presents new difficulties and challenges absent in *in vitro* experiments. Such challenges, i.e., lack of predictability, are based on *in vivo* variables, such as systemic adverse reactions in the subject, efficiency of anti c-kit antibody binding to KIT protein *in vivo*, and variable reactions associated with different inflamed tissue sites. In this application, the success observed in transgenic mice used in the direct therapeutic administration of anti c-kit antibodies on inflamed skin was thus not predictable based on success in an *in vitro* system.

In support of this position, applicant respectfully directs the Examiner to Genovese, et al. ("Combination Therapy with Entaercept and Anakinra in the Treatment of Patients with Rheumatoid Arthritis who have been Treated Unsuccessfully with Methoxtrexate", *Arthritis & Rheumatism*, Vol. 50 No. 5:1412-1419 (2004)) (Exhibit A), Verhoeven, et al. ("Combination Therapy in Rheumatoid Arthritis: Updated Systematic Review", *British Journal of Rheumatology*, 37:612-619 (1998)) (Exhibit B) and the instant specification at page 41, lines 5-32.

Genovese teaches that the *in vivo* combination of two drugs known to individually treat rheumatoid arthritis, etanercept and anakinra, is potentially more harmful to a subject than treatment with either etanercept or anakinra alone. (Genovese, page 1418).

Verhoeven teaches that combination therapy for rheumatoid arthritis, using several drugs known to be capable of treating

the disease individually, produced "no trend for an overall beneficial effect of a particular drug in a combination." (Verhoeven, page 617, Fig. 1). That is, some drug combinations succeeded and some failed, all in unpredictable fashion.

The *in vivo* experiments conducted in Genevese and Verhoeven yielded unexpected results for combination therapy of rheumatoid arthritis, especially in light of the fact that the drugs tested had produced positive results when tested individually in other *in vivo* experiments. It is stressed that in these references *in vivo* data *still* failed to serve as a basis for reasonably expecting success in further *in vivo* experiments. If this is true with *in vivo* data, it only follows that *in vitro* data are an even less reliable indicator of *in vivo* success. In short, one can not reasonably expect *in vivo* success based only on *in vitro* data.

Finally, applicant notes that the specification, at page 41, lines 5-32, makes clear that much skepticism existed in the art as to the predictability of *in vivo* experiments for blocking kit activation based on *in vitro* data. Specifically, it was not clear "what the overall [] effects of blocking KIT would be on inflammation and physiology *in vivo*" (pg. 41, lines 7, 8), based on specific scientific observations enumerated at pg. 41, lines 7-32.

In view of the above remarks, applicant maintains that claims 50-57 satisfy the requirements of 35 U.S.C. §103(a).

Summary

Applicant maintains that the claims pending are in condition for allowance. Accordingly, allowance is respectfully requested.

If a telephone conference would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee is deemed necessary in connection with the filing of this Communication. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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Combination Therapy With Etanercept and Anakinra in the Treatment of Patients With Rheumatoid Arthritis Who Have Been Treated Unsuccessfully With Methotrexate

Mark C. Genovese,[1] Stanley Cohen,[2] Larry Moreland,[3] Deborah Lium,[4] Sean Robbins,[4] Richard Newmark,[4] and Pirow Bekker,[4] for the 20000223 Study Group

Objective. **To determine the potential for additive or synergistic effects of combination therapy with the selective anti–tumor necrosis factor α agent etanercept and the anti–interleukin-1 agent anakinra.**

Methods. **Two hundred forty-four patients in whom rheumatoid arthritis (RA) was active despite methotrexate therapy were treated with subcutaneous etanercept only (25 mg twice weekly), full-dosage etanercept (25 mg twice weekly) plus anakinra (100 mg/day), or half-dosage etanercept (25 mg once weekly) plus anakinra (100 mg/day) for 6 months in a double-blind study at 41 centers in the US. Patients had never previously received anticytokine therapy. Patient response was measured with the American College of Rheumatology (ACR) core set criteria, a health-related quality-of-life questionnaire, and the Disease Activity Score. Safety was assessed by the number of adverse events and clinical laboratory values. Plasma concentrations of both agents and antibody formation against both agents were also assessed.**

Results. **Combination therapy with etanercept plus anakinra provided no treatment benefit over etanercept alone, regardless of the regimen, but was associated with an increased safety risk. Thirty-one percent of the patients treated with full-dosage etanercept plus anakinra achieved an ACR 50% response, compared with 41% of the patients treated with etanercept only. This result was not statistically significant ($P = 0.914$). The incidence of serious infections (0% for etanercept alone, 3.7–7.4% for combination therapy), injection-site reactions, and neutropenia was increased with combination therapy. Combination therapy had no effect on the pharmacokinetics or immunogenicity of either agent.**

Conclusion. **Combination therapy with etanercept and anakinra provides no added benefit and an increased risk compared with etanercept alone and is not recommended for the treatment of patients with RA.**

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by inflammation that often leads to the progressive destruction of articular structures and significant disability. The etiology of RA remains unclear, but it is thought to be mediated in part by antigen-driven T cells and macrophages that produce interleukin-1 (IL-1) and tumor necrosis factor α (TNFα), 2 cytokines involved in the inflammatory cascade (1–3). Specific blockade of these individual cytokines has recently been shown in large, place-controlled trials to be safe and effective in the treatment of RA (4–9).

Although selective anticytokine therapy has improved patient outcomes, it does not necessarily produce disease remission. Thus, the more aggressive treatment option of combining anticytokine agents has been explored in animal studies. Combination treatment with anakinra, a recombinant IL-1 receptor antagonist (IL-1Ra) (Kineret; Amgen, Thousand Oaks, CA), and polyethylene glycol–conjugated soluble TNF receptor type I

Supported by Amgen, Inc., Thousand Oaks, California.

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Drs. Genovese, Cohen, and Moreland have served as consultants for Amgen, Inc.

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esulted in synergistic improvement in the symptoms of djuvant-induced and collagen-induced arthritis in rats elative to the improvement observed with either agent lone (10,11). Additive or synergistic improvement was een with a variety of dose combinations of each agent, ut synergistic improvement was particularly evident hen suboptimal doses of each agent were given in ombination.

The present study was designed to test the hypothesis that combination therapy with the anti-TNF agent etanercept, a soluble TNFα receptor (Enbrel; Amgen), and the anti–IL-1 agent anakinra at their approved dosages would safely provide superior efficacy relative to etanercept alone in patients with RA. Secondarily, the study examined the possibility that anakinra given with etanercept at a dosage of 25 mg once weekly (half the approved weekly dose) would still provide superior efficacy compared with full-dosage etanercept alone.

PATIENTS AND METHODS

Patients. This study enrolled patients who were at least 18 years old and had a >6-month history of RA, as diagnosed by the American College of Rheumatology (ACR) classification criteria (12). Patients had at least 6 swollen joints and 9 tender/painful joints and at least 2 of the following: morning stiffness lasting at least 45 minutes, a serum C-reactive protein (CRP) level of at least 1.5 mg/dl, or an erythrocyte sedimentation rate (ESR) of at least 28 mm/hour. Patients had received methotrexate (MTX) for at least 16 weeks, with the dosage stable at 10–25 mg/week for at least 8 weeks. All patients gave informed consent, and the study protocol was approved by the institutional review boards for each study site.

Patients were not eligible to enroll in the study if they had received any disease-modifying antirheumatic drug other than MTX within the past 4 weeks, had ever been treated with anakinra or any protein-based TNFα inhibitor (e.g., etanercept, infliximab), had received any intraarticular or systemic corticosteroid injections within the past 4 weeks, or had a recent history of significant infection or other important concurrent illness.

Study design and treatment. Patients were randomly assigned in a 1:1:1 ratio to receive 25 mg of etanercept twice weekly plus anakinra placebo once daily, 25 mg of etanercept once weekly plus 100 mg of anakinra daily, or 25 mg of etanercept twice weekly plus 100 mg of anakinra once daily (hereafter referred to a etanercept only, half-dosage etanercept plus anakinra, and full-dosage etanercept plus anakinra, respectively). Both etanercept and anakinra were administered subcutaneously for 24 weeks. In order to blind patients to the treatment assignment, additional sham injections of etanercept were administered as necessary, so that all patients received twice weekly injections of etanercept/sham and once daily injections of anakinra or matched placebo. Patients continued to receive stable doses of MTX and other medications (e.g., corticosteroids) throughout the study. After screening, patients were evaluated at baseline (day 1) and at weeks 2, 4, 8, 12, 16, 20, and 24, with followup evaluation 4 weeks after completion or at the time of early discontinuation.

Endogenous human IL-1Ra was isolated, purified, and produced by recombinant DNA technology using *Escherichia coli* fermentation. The resulting product, anakinra, is identical to the naturally occurring nonglycosylated form of human IL-1Ra except for the addition of an N-terminal methionine residue. Anakinra was provided by Amgen in single-use vials as a liquid containing 1 ml of 100 mg/ml anakinra. The formulation consisted of sodium citrate, sodium chloride, disodium EDTA, and polysorbate 80. The placebo formulation was the same, but without anakinra. Both solutions were pH 6.5.

Etanercept is a soluble TNF receptor fusion protein produced by recombinant DNA technology in a Chinese hamster ovary mammalian cell expression system. Etanercept was supplied by Amgen in 25-mg single-use vials containing etanercept lyophilized powder, mannitol, sucrose, and tromethamine. After reconstitution with bacteriostatic water, the solution had a mean (±SD) pH of 7.4 ± 0.3.

Efficacy assessment. At every study visit, patients were assessed for the components of the ACR core set of disease activity measures (13), the modified Disease Activity Score (DAS) (14), the European League Against Rheumatism (EULAR) response (14), and the duration of morning stiffness. A health-related quality-of-life evaluation with the Short Form 36 (SF-36) healthy survey (15) was performed at baseline and at weeks 4, 12, and 24 (or at the time of early termination).

The primary end point was the proportion of patients achieving an ACR 50% (ACR50) response (16) at week 24. Secondary efficacy end points included the ACR20 and ACR70 response rates at week 24, the ACR response at week 12, the sustained ACR20 response (response for at least 4 monthly measurements, not necessarily consecutive, with 1 occurring at month 6), a good or moderate EULAR response at week 24, improvement in the ACR core criteria components, duration of morning stiffness, the DAS, and the SF-36 score.

Patients were considered ACR50 responders if they had at least a 50% reduction in the number of tender and swollen joints and in 3 of the following 5 measures: patient's assessment of disease activity by visual analog scale (VAS), physician's assessment of disease activity by VAS, patient's assessment of pain by VAS, the disability score as measured by the Health Assessment Questionnaire (17), and acute-phase reactants (CRP or ESR). The joint counts (66 joints evaluated for swelling, and 68 joints evaluated for tenderness/pain) were assessed by the same qualified independent assessors at each study center throughout the study. To preserve blinding of the study, injection sites were covered with clothing during the joint counts to insure that the assessors would not be influenced by injection-site reactions.

Safety and pharmacokinetic assessment. Safety assessment data that were collected at every study visit were the number of adverse events/infectious events and the clinical laboratory values. An adverse event was defined as follows: "Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment" (18). A serious adverse event was defined as follows: "Any untoward medical occurrence that at

Table 1. Baseline characteristics of study patients*

Characteristic	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
% female	82.5	71.6	77.8
% white race	86.3	77.8	75.3
Age, years	54.4 ± 13.6	53.8 ± 11.8	55.7 ± 13.0
% age ≥ 65 years	25.0	18.5	29.6
Weight, kg	75 ± 18	82 ± 21	80 ± 23
Duration of RA, years	9.7 ± 9.4	9.5 ± 10.3	10.6 ± 9.8
% rheumatoid factor positive	65.0	75.3	71.6
% receiving corticosteroids	48.8	54.3	44.4
NSAID use, %	96.3	95.1	96.3
MTX dosage, mg/week	16.1 ± 4.5	16.2 ± 4.2	15.7 ± 5.0
No. of tender/painful joints	31.0 ± 14.2	31.0 ± 15.4	35.9 ± 14.9
No. of swollen joints	21.4 ± 9.4	19.8 ± 9.6	23.4 ± 12.0
HAQ score	1.5 ± 0.6	1.5 ± 0.6	1.6 ± 0.6
Serum CRP, mg/dl	2.0 ± 2.2	2.4 ± 3.5	2.0 ± 2.5
ESR, mm/hour	44.6 ± 21.51	49.2 ± 22.81	49.9 ± 23.94
Duration of morning stiffness, minutes	145.3 ± 102.3	154.4 ± 162.1	159.5 ± 134.0
SF-36 score			
Physical component	28.7 ± 9.7	28.8 ± 7.9	29.1 ± 7.9
Mental component	46.9 ± 12.3	47.9 ± 10.9	44.5 ± 11.9

* Except where indicated otherwise, values are the mean ± SD. Etanercept only = etanercept 25 mg twice weekly; half-dosage etanercept + anakinra = etanercept 25 mg once weekly plus anakinra 100 mg daily; full-dosage etanercept + anakinra = etanercept 25 mg twice weekly plus anakinra 100 mg daily; RA = rheumatoid arthritis; NSAID = nonsteroidal antiinflammatory drug; MTX = methotrexate; HAQ = Health Assessment Questionnaire; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; SF-36 = Short Form 36.

any dose results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect" (18).

Blood samples were collected for measurement of plasma anakinra and etanercept concentrations (at baseline and weeks 4, 12, and 24 [or at the time of early termination]) and anti-anakinra and anti-etanercept antibodies (at baseline and weeks 12 and 24 [or at the time of early termination]). Plasma anakinra concentrations were assessed by antibody-capture enzyme-linked immunoassay (ELISA), and plasma etanercept concentrations were assessed by a solid-phase sandwich ELISA kit. Antibody samples testing positive in a screening biosensor immunoassay (Biacore, Uppsala, Sweden) were retested for neutralizing antibodies, using a bioassay.

Statistical analysis. Results were analyzed using a modified intent-to-treat method that included all randomized patients who received at least 1 dose each of anakinra/placebo and etanercept/sham. Patients with missing ACR scores at a particular visit were considered nonresponders at that visit.

The primary comparison was the full-dosage etanercept plus anakinra group against the etanercept-only group. The analysis was 1-tailed for the primary comparison and 2-tailed for the secondary comparisons. Odds ratios (ORs) and their confidence intervals (CIs) were calculated for comparisons between groups. Binary efficacy end points were analyzed using a logistic regression model. Continuous end points were analyzed over time using a repeated-measures mixed model.

Adverse events were tabulated for comparison across treatment groups, and summary statistics were calculated for laboratory values.

RESULTS

Characteristics of the study patients. The baseline demographics and disease characteristics of the patients are shown in Table 1. Most of the patients were women with long-standing and very active disease. Characteristics were balanced across treatment groups. Com-

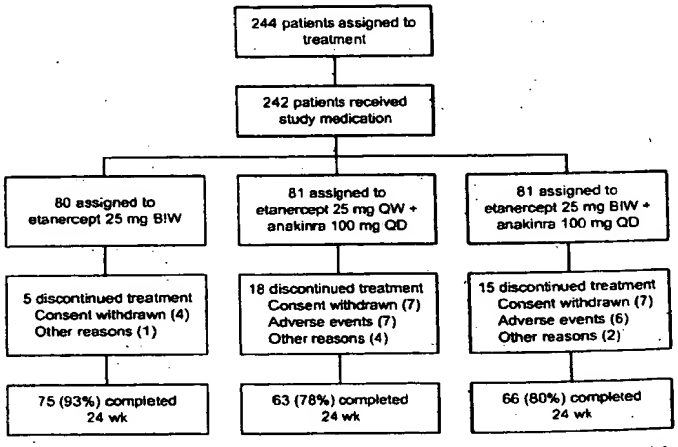

Figure 1. Disposition of patients. Other reasons for premature withdrawal included protocol violations and patients being lost to followup. BIW = twice weekly; QW = once weekly; QD = once daily.

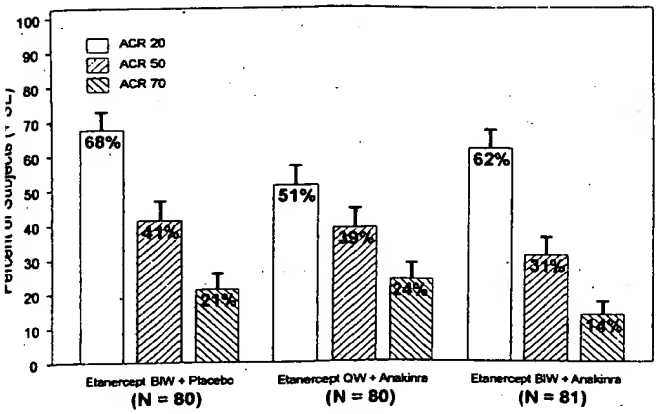

Figure 2. Percent of patients achieving an American College of Rheumatology 20% (ACR20), ACR50, or ACR70 response at week 24. BIW = twice weekly; QW = once weekly.

pletion rates ranged from 78% to 93%, with the highest rate in the etanercept-only group (Figure 1). Significantly fewer patients in this group withdrew as a result of adverse events compared with the combination-therapy groups.

Efficacy results. Patients in all treatment groups showed improvement from baseline at week 24 (Figure 2). Therapy in the etanercept-only group resulted in an ACR50 of 41%, compared with 31% in the full-dosage etanercept plus anakinra group ($P = 0.914$, by 1-tailed χ^2-test). The OR for achieving an ACR50 response in the full-dosage etanercept plus anakinra group relative to that in the etanercept-only group was 0.64 (90% CI 0.37–1.09). The OR for achieving an ACR50 response in the etanercept-only group relative to that in the half-dosage etanercept plus anakinra group was 1.11 (95% CI 0.59–2.09). For the comparison of patients in the full-dosage (twice weekly) combination-therapy group relative to those in the low-dosage (once weekly) combination therapy group, the OR was 0.71 (95% CI 0.37–1.35).

Because the dropout rate was higher in the groups receiving combination therapy, it was important to determine whether this influenced the efficacy results. Sensitivity analyses, including a completers analysis (all patients who completed the study) and a last observation carried forward analysis, yielded results similar to those observed in the modified intent-to-treat analysis (data not shown), indicating that differential dropout rates did not influence the outcome. In addition, results were unaffected when they were adjusted for baseline covariates (data not shown).

Evaluation of the ACR20 and ACR70 response rates confirmed that combination therapy was not superior to etanercept alone. The only comparison yielding a statistically significant difference between treatments indicated that at week 24, the ACR20 response of patients treated with etanercept alone was superior to that of patients treated with etanercept once weekly plus anakinra (OR 1.98, 95% CI 1.05–3.78; $P = 0.037$).

Between 43% and 54% of patients in each treatment group achieved a sustained ACR20 response during the study, and most patients achieved a EULAR response at week 24 (79% of patients in etanercept-only group, 73% of those in the full-dosage etanercept plus anakinra group, and 66% of patients in the half-dosage etanercept plus anakinra group received a good or moderate rating). At week 24, the mean percent reduction from baseline in the DAS was 39% in the

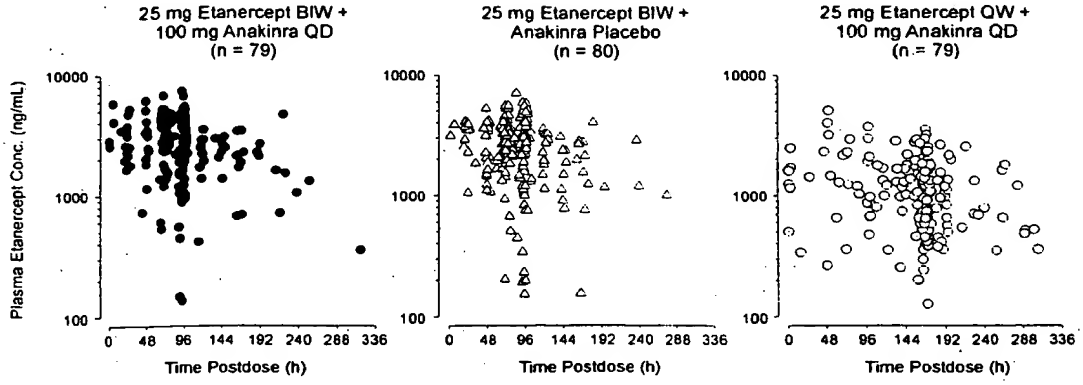

Figure 3. Individual plasma etanercept concentrations (Conc). Because the results at each week indicated that the pharmacokinetic steady state of etanercept was reached by week 4, the data for all visits (weeks 4, 12, and 24) were pooled for analysis. BIW = twice weekly; QW = once weekly; QD = once daily; h = hours.

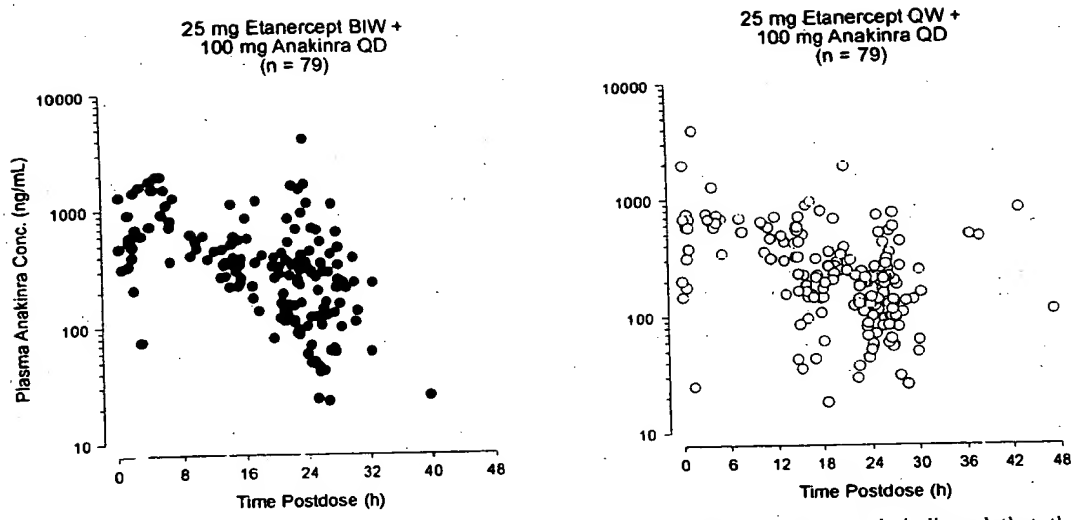

Figure 4. Individual plasma anakinra concentrations. Because the results at each week indicated that the pharmacokinetic steady state of anakinra was reached by week 4, the data for all visits (weeks 4, 12, and 24) were pooled for analysis. See Figure 3 for definitions.

etanercept-only group, 40% in the half-dosage etanercept plus anakinra group, and 41% in the full-dosage etanercept plus anakinra group. Radiographs were not obtained in this study.

Pharmacokinetics. Plasma concentrations for weeks 4, 12, and 24 indicated that the pharmacokinetic steady state was reached by week 4 for both etanercept and anakinra, and thus the data for all visits were pooled for analysis. The pharmacokinetics of each agent appeared unaffected by the concomitant administration of the other (Figures 3 and 4). Plasma concentrations of anakinra and etanercept were similar to those observed in previous studies of anakinra or etanercept alone (Genovese MC, et al: unpublished observations).

Safety. The proportion of patients reporting adverse events was similar for each treatment group (90–95%) (Table 2). However, combination therapy with anakinra and etanercept was associated with a higher overall incidence of serious adverse events, events causing patient withdrawal from study, and injection-site reactions.

Serious adverse events were generally individual occurrences, with no single category of event accounting for the increase observed with combination therapy

Table 2. Incidence of adverse events during treatment*

Event	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
Any adverse event	72 (90.0)	77 (95.1)	76 (93.8)
Most common adverse events			
Injection-site reaction	32 (40.0)	55 (67.9)	57 (70.4)
Upper respiratory infection	16 (20.0)	9 (11.1)	11 (13.6)
Any adverse event causing withdrawal	0 (0)	7 (8.6)	6 (7.4)
Any serious adverse event	2 (2.5)	4 (4.9)	12 (14.8)
Any infection	32 (40.0)	30 (37.0)	38 (46.9)
Infection resulting in antibiotic administration or hospitalization	0 (0.0)	3 (3.7)	6 (7.4)
Infection leading to withdrawal	0 (0.0)	2 (2.5)	2 (2.5)
Serious infection	0 (0)	3 (3.7)	6 (7.4)
Serious pneumonia	0 (0)	1 (1.2)	2 (2.5)
Serious cellulitis	0 (0)	1 (1.2)	2 (2.5)

* Values are the number (%) of patients. See Table 1 for definitions.

Table 3. Serious adverse events that occurred during the study*

Event	Etanercept only (n = 80)	Half-dosage etanercept + anakinra (n = 81)	Full-dosage etanercept + anakinra (n = 81)
Cellulitis	0 (0)	1 (1.2)	2 (2.5)
Pneumonia	0 (0)	1 (1.2)	1 (1.2)
Pneumonia and pulmonary fibrosis leading to respiratory insufficiency	0 (0)	0 (0)	1 (1.2)
Gastroenteritis	0 (0)	0 (0)	1 (1.2)
Herpes zoster	0 (0)	0 (0)	1 (1.2)
Lymphoma, malignant	0 (0)	0 (0)	1 (1.2)
Neuralgia	0 (0)	0 (0)	1 (1.2)
Back pain	0 (0)	0 (0)	1 (1.2)
Chest pain, cardiac	0 (0)	0 (0)	1 (1.2)
Chest pain, noncardiac	0 (0)	0 (0)	1 (1.2)
Pyelonephritis	0 (0)	0 (0)	1 (1.2)
Transient ischemic attack	0 (0)	0 (0)	1 (1.2)
Arrhythmia, atrial	1 (1.3)	0 (0)	0 (0)
Dyspnea	0 (0)	1 (1.2)	0 (0)
Gastric ulcer, hemorrhage	0 (0)	1 (1.2)	0 (0)
Personality disorder	1 (1.3)	0 (0)	0 (0)
Pneumonitis	0 (0)	1 (1.2)	0 (0)

* Values are the number (%) of patients. See Table 1 for definitions

(Table 3). However, infections accounted for serious events in 9 of 16 patients receiving combination therapy. The reported serious infections were as follows: pneumonia and cellulitis (3 patients each), herpes zoster (1 patient), pneumonitis (1 patient), and pyelonephritis (1 patient). One 70-year-old patient with pulmonary fibrosis was diagnosed as having pneumonia and died of pulmonary insufficiency. Serious infections occurred an average of 2 months after exposure to combination treatment (range 1 week to 5 months) in patients whose mean age was 60 years (range 42–77 years). No cases of tuberculosis or opportunistic infections were reported.

The incidence of injection-site reactions was more than 50% higher with combination therapy than with etanercept alone. These reactions were transient, rarely severe (a combined incidence of 2% in the combination-therapy groups), and typically involved no clinical sequelae. However, they were the most common cause of adverse event–related withdrawal during the study for patients receiving combination therapy, accounting for 5 of 13 adverse event–related withdrawals. Injection-site reactions were less likely to occur after the first month of therapy.

The mean neutrophil counts decreased to a similar extent in all 3 groups within the first 2 weeks of initiating treatment but remained stable thereafter. Two patients in the full-dosage etanercept plus anakinra group experienced neutropenia (neutrophil count $<1.0 \times 10^9$/liter) during the study. Both patients completed the study, and no clinical events were associated with the neutropenia. No subjects had changes in the neutrophil count that appeared to be associated with their serious infectious episodes. None of the subjects who experienced serious infectious episodes experienced neutropenia. No other clinically significant trends in the laboratory results were apparent. Three patients receiving anakinra and no patients receiving etanercept had evidence of potentially neutralizing anti-anakinra and anti-etanercept antibodies, respectively. The presence of antibodies had no apparent effect on efficacy or safety.

DISCUSSION

The clinical hypothesis for this study, that combination treatment with selective anticytokine therapies (etanercept and anakinra) would safely provide superior improvement in the signs and symptoms of RA compared with etanercept alone, proved false. Analyses of the primary efficacy end point, the ACR50 response at week 24, showed no significant differences between groups regardless of the combination regimen used (etanercept twice weekly or once weekly). In fact, patients who received etanercept only in this study had the highest ACR responses, similar to those seen in previous studies of etanercept (4,8), and encountered the fewest safety problems.

Results of preclinical experiments suggested that simultaneous blockade of IL-1 and TNFα would be more effective than either approach alone in inhibiting

progression of RA, with a combination of suboptimal doses of each anticytokine agent providing a synergistic effect. The reasons for the discrepancy between the preclinical and clinical results are unclear, but several theories present themselves. First, there may have been a negative interaction between the compounds. This theory seems unlikely, because the pharmacokinetic results show that plasma concentrations for both agents remained unchanged relative to observations for each agent independently. Also, anakinra is eliminated renally (19–21), while etanercept is eliminated by the Kupffer cells of the liver (Genovese MC, et al: unpublished observations). It is also unlikely that anakinra and etanercept would bind and block the effect of each other.

Second, anti-anakinra antibodies could be responsible for the lack of benefit of combination therapy. This too is unlikely, because a low percentage of patients had potentially neutralizing anti-anakinra antibodies, and the antibody results from this study were consistent with the antibody data from other anakinra studies in which efficacy was demonstrated. Third, anti-TNF therapy could have down-regulated IL-1 expression, rendering any impact of anti–IL-1 therapy negligible. This remains a possibility, although it would not explain the inconsistency between the clinical and preclinical results. Fourth, the degree of overlap and interplay between IL-1 and TNF may leave little room for improvement beyond the efficacy attainable with an effective TNF inhibitor. This is an intriguing possibility that requires further study.

The safety results for this study showed that combination anticytokine therapy was associated with a higher incidence of serious infections than was observed with the use of etanercept alone. These results are comparable with what was previously reported in a small open-label study of the combination of etanercept and anakinra (22). Many patients in this study were receiving a combination of 4 potentially immunosuppressive agents, including corticosteroids, MTX, anakinra, and etanercept, all of which could have contributed to this finding. Combination therapy was also more commonly associated with neutropenia, which is probably not surprising because decreases in the neutrophil count have been associated with both etanercept and anakinra (Genovese MC, et al: unpublished observations). It is notable, however, that in this study neutropenia was not associated with the risk of serious infection.

These results suggest that use of combination treatment with anakinra and etanercept is not justified in patients with RA who are naive to biologic therapy. However, the possibility that combination anticytokine treatment could benefit certain patients cannot be excluded based on this relatively small study. The response to combination therapy might be different in patients with partial or inadequate responses to prior anticytokine treatment, for example, although the safety concerns raised by this study would remain. Furthermore, these results do not preclude the possibility of successful combination therapy with future agents selectively blocking other pathways.

Overall, the results from this study provide no evidence of an additional treatment benefit of combination therapy with etanercept plus anakinra in patients with active RA despite the use of MTX. In fact, the combination posed an increased risk of serious infection and neutropenia. Treatment with etanercept or anakinra alone, or either agent in combination with MTX, has been demonstrated to be effective and safe in previous studies, and the findings from this study do not affect the profiles of the individual compounds. The findings do raise important questions about the role of each of these cytokines in the pathophysiology of RA and other inflammatory diseases. The development of anticytokine therapies has epitomized the translation of research from bench to bedside, and the results of this study highlight the need to translate these results back to the bench to better understand the interrelationship of these cytokines in human disease and to explain the incongruity of the preclinical and clinical findings.

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APPENDIX A: MEMBERS OF THE 20000223 STUDY GROUP

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DISEASE-MODIFYING DRUGS
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COMBINATION THERAPY IN RHEUMATOID ARTHRITIS: UPDATED SYSTEMATIC REVIEW

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SUMMARY

In a second update of a systematic review, many new developments in the combined drug treatment of rheumatoid arthritis (RA) are highlighted. In early RA patients, step-down bridge therapy that includes corticosteroids leads to much enhanced efficacy at acceptable or low toxicity. The effects on joint damage may be persistent, but the symptomatic effects are probably dependent on continued corticosteroid dosing. In late patients, cyclosporin improves a suboptimal clinical response to methotrexate, and the triple combination of methotrexate, sulphasalazine and hydroxychloroquine appears to be clinically better than the components. Other combinations are either untested, tested at low sample size, or show negative interaction. In view of the low volume of evidence, most studies need confirmation by replication.

KEY WORDS: Rheumatoid arthritis, Combined treatment, DMARDs, Glucocorticoids, Systematic review.

THERE is a trend among rheumatologists to treat rheumatoid arthritis (RA) patients earlier and more aggressively. New scientific evidence supports early intervention with disease-modifying anti-rheumatic drug (DMARD) therapy [1]. Rapid and adequate control of disease activity is aimed at the prevention of structural joint damage and subsequent loss of function and quality of life. In this setting, combining so-called DMARDs might lead to additive effects. Alternatively, doses might be reduced, and perhaps some of the toxicity avoided. Many rheumatologists already prescribe combination therapy, although until recently scientific evidence to support this policy was lacking. Over the last few years, an increasing number of high-quality trials have been published. We present a second update of a systematic review of combination therapy in RA [2, 3].

In combining DMARDs, three main strategies can be distinguished. In this review, the label 'step-up strategy' is reserved for trials in which patients with insufficient clinical benefit from one second-line agent continued the use of this first drug and had another (or placebo) added to this. The label 'parallel' was assigned to trials in which the patients started with a combination of new drugs, and 'step-down' to trials with sequential withdrawal of simultaneously started drugs, prescribed by protocol.

METHODS

Study identification

The MEDLINE database was searched from August 1992 (the closing date of the previous review) to July 1997 using the MeSH headings: 'arthritis, rheumatoid'; and 'drug therapy, combination'. The bibliographies of all retrieved articles were scrutinized for additional studies. The first authors of studies published only in abstract form were contacted. Such studies were eligible for inclusion if a full manuscript was available. Titles and abstracts (when available) were screened by one author (MB up to August 1992, ACV subsequently) and any article in English, French, German or Dutch that appeared potentially relevant was retrieved.

Study selection and validity assessment

First, the quality of the studies, and thus the strength of evidence, was scored on a three-point scale on the basis of two primary criteria: randomization and blinding. Accordingly, strong evidence came from randomized, double-blind studies; moderately strong evidence from studies that were randomized, but open or partially blinded; and weak evidence from all other studies. This score specified the maximum strength we felt a study could yield. A second set of criteria, modified from Sackett *et al.* [4] was then applied. These were: (a) adequate outcome assessment (blind and comprising toxicity); (b) adequate description of study patients (report of at least age and sex, some record on the previous disease severity and concurrent medication); (c) adequate description of the therapeutic manoeuvre (i.e. minimal potential of bias, with blinding, contamination, co-intervention and compliance properly addressed), (d) complete accounting of study patients in the results. To obtain the final quality score, points were subtracted for each of these criteria not met.

Data extraction and analysis

The results of the trials yielding moderately strong or strong evidence in the previous reviews (original and update) were added to the information from the new search. Data extracted from the selected studies

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included baseline patient characteristics, study and concomitant treatment, outcome measures, and details on toxicity, withdrawals and eligibility criteria for disease activity.

Clinical efficacy, i.e. improvement in clinical outcome measures, was compared between the combined-treatment group and the single-treatment group. In the case of more than one control group, comparisons were made between the combined-treatment group and each control group, but eventually the comparison with the best performing control group was decisive. The WHO/ILAR core set measures assessed efficacy [5]. These measures comprise tender and swollen joint count (or a score), pain assessment, patient and physician (or observer) global assessment, physical function index [here, in every case Health Assesement Questionnaire (HAQ) or a modification thereof], and acute phase reaction [i.e. erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP)]. When less than four of these measures had been assessed, first grip strength and second morning stiffness were selected as well. Four levels of efficacy were distinguished based on differences in improvement in the selected measures:

- combined treatment 'substantially more effective' (' + + ' in the summary table): significantly greater improvement in the combined-treatment group in at least half of the selected measures (minimum two out of four), plus improvement of at least 150% that of the control group;
- combined treatment 'more effective' (' + '): significantly greater improvement in at least half of the selected measures;
- 'positive trend' (' + ?'): significantly greater improvement in at least 25% of the measures, or significantly greater improvement only in a predefined summary index of measures;
- 'no difference' (' = '), the remainder.

When the total number of core set measures (plus grip strength and morning stiffness) was less than four, only trends were scored.

Toxicity was rated as increased (' + ') when significantly more patients from the combined-treatment group were withdrawn from the study medication because of adverse events. Likewise, it was rated as decreased (' − ') when significantly less patients from the combined-treatment group were withdrawn from the study medication because of adverse events. A significant difference (or trend) in numbers of adverse events not leading to withdrawal was rated as 'trend of more toxicity' (' + ?') or 'trend of less toxicity' (' − ?'). Where possible, results of statistical tests comparing the effect or toxicity of the different treatments were calculated or recalculated using the reported data.

RESULTS

Previous work had yielded eight relevant studies [2, 3]. Six of these eight provided 'strong' or 'moderately strong evidence' and are included in the final selection [6, 12, 14, 20, 24, 26]. The current search, covering the interval between August 1992 and July 1997, yielded 231 new citations. Together with previous reviews, this brings the total to 611 titles scanned, 100 were retrieved and 18 selected for review. Of the screened abstracts and titles, 38 were linked to a possibly relevant article. Three articles in Japanese [27–29] were not rated. Not selected articles were case reports, editorials, observational or non-randomized studies. Three studies that described adjuvant treatment with oral corticosteroids, androgens and oestrogens, respectively, were not included because they failed to meet the criterion of a one-type single DMARD control group [30–32]. Two studies described an extended follow-up or radiological assessments of an already selected article [33, 34]; the data from these publications were added to those of the original study [19, 20]. Two articles were found in the reference list of selected articles [9, 10]. Four possibly relevant reports in abstract form were found in abstract book supplements. The corresponding manuscripts of two recently published articles were obtained [21, 22].

The total of 20 (six old, 14 new) included trials are listed in Table 1. The total number of patients included in these trials is 1952. All trials used a more or less strict criterion to verify the presence of active disease. The studies are ranked according to treatment strategy as well as the DMARDs of choice. Six studies describe a step-up strategy; two of these used cyclosporin, three used i.m. gold as anchor drug, and two methotrexate. Ten studies describe a parallel strategy; of these, six used methotrexate (all but one as anchor drug), six studies used antimalarials (one as anchor drug), three used sulphasalazine, one i.m. gold, dapsone or D-penicillamine (as anchor drugs); also used were auranofin and azathioprine (as additional drug; total more than 10, due to combinations). The studies with a step-down strategy (four in total) all used steroids [i.m. methylprednisone pulses or prednis(o)one orally]. Steroids were added to i.m. gold (in two studies) or sulphasalazine (also in two studies; in one study prednisolone was added together with methotrexate).

Studies with step-up strategy

Smyth *et al.* [6] added 75 mg/day cyclophosphamide or placebo to a stable and continued pre-trial dose of prednisone varying between 3 and 15 mg/day in 29 patients with established disease. After 6 months, outcomes in the combined-treatment group were significantly more improved in grip strength and an inflammatory index comprising swelling, redness, pain on motion, heat and tenderness, but not in ESR. Only one case of alopecia was reported in the combined-treatment group with no withdrawal due to toxicity in either group. Given the paucity of outcomes, this suggests a trend of increased efficacy with no increase in toxicity, but the disease activity at baseline was less in the placebo group.

Moreland *et al.* [7] performed a dose-finding study of monoclonal anti-CD4 antibody cM-T412 in three different doses or placebo added to stable treatment with methotrexate (≤ 15 mg/week) in 64 patients with

TABLE I

Reviewed trials clustered by drug combination and combination strategy

First author	Ref.	Publication year	n patients	n groups	Compared drugs or combinations	Therapy strategy	Strength of evidence	Disease duration (yr)	Assessments at (month)	'C' score	Efficacy	Toxicity
Smyth	[6]	1975	29	2	(Pred CyP) vs Pred	Step-up	Moderate	>2	6	2/3	+	=
Moreland	[7]	1995	64	4	(MTX αCD4) vs MTX	Step-up	Strong	9	3	0/6	=	=
Tugwell	[8]	1995	148	2	(MTX CyA) vs MTX	Step-up	Strong	10	6	6/7	++	=
Rendis	[9]	1996	40	2	(AU CyA) vs AU	Step-up	Strong	11	6	1/7*	=	+?
Yasuda	[10]	1994	24	2	(AU Dnc) vs AU	Step-up	Strong	8	3	3/4	+	+?
Porter	[11]	1993	142	2	(AU Hcq) vs AU	Step-up	Moderate	6	6	0/4	=	=
Scott	[12]	1988	101	2	(AU Hcq) vs AU	Parallel	Strong	2	12	1/11	-?	+?
Fiarvang	[13]	1993	91	3	(SSZ Hcq) vs SSZ vs Hcq	Parallel	Strong	7	6	0;2/5	=	=
Gibson	[14]	1987	72	3	(Dncn Cq) vs Dncn vs Cq	Parallel	Moderate	2	12	0;0/5	=	=
Haar	[15]	1993	80	3	(Daps Hcq) vs Daps vs Hcq	Parallel	Strong	2	6	0;0/4	=	=
Trnavsky	[16]	1993	40	2	(Hco MTX) vs Hcq	Parallel	Strong	>2	6	2/5	+?	+?
Ferraz	[17]	1994	82	2	(MTX Cq) vs MTX	Parallel	Strong	3	6	2/4	+	+?
O'Dell	[18]	1996	102	3	(MTX SSZ Hcq) vs (SSZ Hcq) vs MTX	Parallel	Strong	9	9	2;2/5	+?	=
Williams	[19]	1992	235	3	(MTX AUR) vs MTX vs AUR	Parallel	Strong	5	12	0;0/5	=	+?
Wilkens	[20]	1992	209	3	(MTX AZA) vs MTX vs AZA	Parallel	Strong	8	12	0;2/6	=	+?
Haagsma	[21]	1997	105	3	(MTX SSZ) vs MTX vs SSZ	Parallel	Strong	<1	6	0;0/7	=	=
Boers	[22]	1997	151	2	(SSZ MTX Pred) vs SSZ	Step-down	Strong	<1	6	6/7	++	-
van Gestel	[23]	1995	40	2	(AU Pred) vs AU	Step-down	Strong	2	3	1/6	++	=
Corkill	[24]	1990	59	2	(AU MP) vs AU	Step-down	Strong	6	3	3/4	++	+?
Cicconetti	[25]	1996	38	2	(SSZ MP) vs SSZ	Step-down	Moderate	6	6	0/4	=	=

'C' score is the number of clinical WHO/ILAR core set outcome measures (see Methods) significantly better in the combined-treatment group in comparison with control group(s). Results from comparisons with two control groups are separated by a semicolon (;). Behind the slash () is the total number of assessed core set measures. NB. The efficacy rating is derived from the C score, and in some cases also from improvement in two non-core set measures (grip strength and morning stiffness) or from improvement in a predefined primary outcome index.

AU, im gold salts; AUR, auranofin; AZA, azathioprine; Bnc, bucillamine; αCD4, monoclonal anti-CD4 antibodies (three doses); Cq, chloroquine; CyA, cyclosporin; CyP, cyclophosphamide; Dapt, dapsone; Dpen, D-penicillamine; Hcq, hydroxychloroquine; MP, methylprednisone pulse; MTX, methotrexate; Pred, prednisone; Pred., prednisolone; SSZ, sulphasalazine.

*Non-core set measures of patient's and (non-blind) clinician's assessment of overall efficacy did show significantly more improvement in the combined-treatment group.

†The combined-treatment group showed significantly better scores for a validated disease activity index based on five variables in a flow chart.

‡Low sulphasalazine doses in the combined-treatment group: 1 g/day.

§Low methylprednisone doses: 5 mg/cg/pulse.

efractory RA. Assessments after 3 months treatment nd 4 i.v. pulses of anti-CD4 did not show any relevant etween-group difference in clinical efficacy or toxicity.

Tugwell *et al.* [8] added cyclosporin or placebo to nethotrexate in 148 patients with established disease nd insufficient response to methotrexate alone. After 5 months, all outcomes with the exception of ESR were substantially and significantly better in the combined-treatment group (HAQ and global assessments $P < 0.001$). Expressed in percentages, improvement as compared with placebo varied between 19 and 26%. The frequency of adverse effects was similar to prior trials of methotrexate and cyclosporin used alone. A threshold of 30% increase in serum creatinine for dose reduction resulted in a relatively low mean cyclosporin dose (3 mg/kg). Eighty per cent of the included patients had stable co-medication with low-dose corticosteroids (≤ 10 mg).

Dendix and Bjelle [9] added cyclosporin or placebo to i.m. gold treatment in 40 patients. After 6 months the combined treatment showed increased efficacy only in patient's global assessments of overall health and clinical efficacy, and non-blind assessments by a treating physician ($P < 0.01$ and < 0.05); other core set measures, including blinded observer's global assessment, showed no difference. No serious adverse effects were noted. Higher blood pressure and signs of renal function impairment were found more often in the cyclosporin-treated group, also dose reduction was required significantly more often in the cyclosporin group. Adverse events requiring symptomatic treatment occurred only in eight patients, four in each treatment group. Six months after the end of combination therapy, all differences had disappeared.

Yasuda *et al.* [10] added bucillamine, a drug developed in Japan, or placebo to i.m. gold treatment in 24 patients. After 3 months, the combined-treatment group had significantly better outcomes in swollen joint count, physician's global assessment and CRP ($P < 0.05$), and similar outcomes (trend) in tender joint count and ESR. Withdrawal for lack of efficacy only occurred in the control group (five patients), and withdrawal due to toxicity occurred more often in the combined-treatment group (5 vs 3).

Porter *et al.* [11] added hydroxychloroquine or placebo to i.m. gold treatment in 142 patients. After 6 months, no differences were evident between the groups. Withdrawal (for all reasons) was comparable in both trial groups ($\sim 28\%$). Owing to the lack of description of previous medication and patient compliance, the strength of evidence was rated as moderate.

Studies with parallel strategy

Scott *et al.* [12] also tested the combination hydroxychloroquine and i.m. gold, but in a parallel strategy against i.m. gold alone in 101 patients. After 12 months of treatment, the combination showed a positive trend in all of the outcomes, but only CRP ($P = 0.01$) and the *a priori* defined composed summary index of disease activity were significantly better ($P < 0.05$). There was less progression of joint damage on radiographs in the combined-treatment group, although this did not reach significance. The total withdrawal rate was high: 42%. The authors report that toxicity might be enhanced as 18 patients in the combined-treatment group *vs* 10 in the control group were withdrawn for adverse effects.

Faarvang *et al* [13] compared the combination of sulphasalazine and hydroxychloroquine with each of these agents alone in 91 patients. Analysis of study completers after 6 months treatment showed no difference between combined treatment and single sulphasalazine treatment. However, combined treatment did show better outcomes in swollen joint count and patient's global assessment compared to hydroxychloroquine alone ($P < 0.05$). In our view, this only confirms that sulphasalazine is a more effective drug than hydroxychloroquine. Both groups showed similar progression of joint damage on radiographs. Withdrawal, for adverse effects as well as other reasons, in this trial was frequent in all treatment groups (32%).

Gibson *et al.* [14] compared the combination of D-penicillamine and chloroquine in comparison with each of these alone in 72 patients. After 12 months, the decreases in ESR in the combined treatment group were significantly larger compared to chloroquine, but not compared to D-penicillamine. Improvements in morning stiffness, joint tenderness and swollen joint score and grip strength did not show significant contrasts between treatment groups. There were significantly more adverse effects in the combined-treatment and the D-penicillamine groups compared with chloroquine.

Haar *et al.* [15] compared the combination of hydroxychloroquine and dapsone with each of these drugs alone in 80 patients. After 6 months of treatment, the combination showed a positive trend with a significant difference only in one measure: ESR, but the baseline values for ESR were also better in this treatment group. Patients treated with combined dapsone and hydroxychloroquine showed less progression of joint damage, but this result was weakened by serious disbalance between groups in baseline values. Withdrawals for toxicity were more numerous in the combined-treatment group, but this difference was not significant (8 *vs* 3 and 4; $P = 0.11$).

Trnavsky *et al.* [16] compared the combination of hydroxychloroquine and methotrexate with hydroxychloroquine and placebo in 40 patients. After 6 months, the combined treatment showed a positive trend with significantly better outcomes in two of five core set measures; patient's global assessment and ESR ($P < 0.05$). The combined-treatment group contained more patients without progression of joint damage, but the report allows no conclusion on whether this difference was significant. Withdrawal for adverse effects was rare (one case).

Ferraz *et al.* [17] compared the combination of methotrexate and chloroquine to methotrexate alone in 82 patients. After 6 months, the combination was more effective in tender joint count ($P = 0.04$) and HAQ ($P = 0.04$) The authors state that combined treatment was slightly more toxic (and effective),

although only three patients were withdrawn due to adverse effects (two of whom had combined treatment). The percentage loss to follow-up (partially related to non-compliance) was quite high (9%) in this study.

O'Dell *et al.* [18] compared the combination of methotrexate, sulphasalazine and hydroxychloroquine to the combination of sulphasalazine and hydroxychloroquine, and to methotrexate alone, in 102 patients. The dose of sulphasalazine (1 g/day) was low. Every 3 months, dose adjustments of methotrexate were allowed, guided by assessments of the effect of therapy. The main assessment of efficacy was after 9 months when no further opportunity was offered to adjust the methotrexate dose in case of insufficient therapy response (by definition <50% improvement in modified Paulus criteria). At this time, 27/31, 23/35 and 28/36 patients were considered responders (χ^2 test: triple therapy *vs* sulphasalazine–hydroxychloroquine $P = 0.04$; *vs* methotrexate $P = 0.32$; overall $P = 0.12$). Based on survival analyses, the authors report significantly more patients with a response to triple therapy after 9 months and conclude that triple therapy results in enhanced efficacy with no increase in toxicity. Non-responders were considered therapy failures and further report on follow-up was restricted to responders. At 9 months of follow-up and according to the rules of this review, triple therapy only showed a positive trend: significantly better swollen and tender joint counts compared to methotrexate, and significantly better swollen joint count and ESR compared to hydroxychloroquine–sulphasalazine. At 2 yr follow-up (with 38% patients still in follow-up), the between-treatment group contrasts were larger (and highly significant), but this concerns only the patients who had a sufficient response according to the modified Paulus criteria at month 9. Withdrawal for toxicity was rare at year 2, but data on withdrawals due to adverse events at month 9 were not available; however, overall withdrawal at month 9 was about equal.

Williams *et al.* [19] compared the combination of methotrexate and oral gold ($n = 106$) against treatment with each of these agents alone in 335 patients. After 48 weeks, none of the five assessed core set measures showed more benefit in the combined-treatment group. Withdrawals for toxicity occurred somewhat more frequently in the combined-treatment group (21% *vs* 15% and 14%; trend, not significant). Subsequently, Lopez-Mendez *et al.* [33] reported no differences between the groups in progression of radiographic scores at week 48.

Willkens *et al.* [20] compared the combination of methotrexate and azathioprine with each of these drugs alone in 209 patients. Data on ESR and HAQ were subsequently added in a letter [35], and data on 48 weeks of follow-up and radiological progression were published later [34]. The combination was not better in the between-group comparisons (withdrawals considered as treatment failures), except for ESR when combined treatment was compared to single azathioprine ($P = 0.03$). The authors also report a trend of less radiographic progression in the methotrexate group. Adverse effects occurred primarily in the combined treatment and azathioprine group (trend). Numbers of withdrawn patients per treatment group due to toxicity are not available, but therapeutic interventions related to adverse events were reported more often in the combined treatment group (48% *vs* 25% and 21%).

Haagsma *et al.* [21] compared the combination of methotrexate and sulphasalazine with each of these drugs alone in 105 patients. After 52 weeks, the combination was not more effective in any of the four core set or other measures. Response to treatment was exceptionally good in all groups: 74% met the preliminary ACR criteria for improvement [36]. Fewer patients were withdrawn for toxicity reasons in the single methotrexate treatment group compared to the combined-treatment group ($P = 0.025$). In contrast with most other trials, the patients included in this study had early disease. These results agree with those of a trial only published in abstract form [37], but contrast with the results of an open trial (not included in this review) in which patients with insufficient reaction to sulphasalazine first stopped this drug and afterwards were randomized to combined methotrexate–sulphasalazine, or methotrexate alone. Here, the combined-treatment group showed significantly better outcomes [38].

Studies with step-down strategy

Boers *et al.* [22] compared the combination of sulphasalazine (2 g/day), methotrexate (7.5 mg/week) and prednisolone (initially 60 mg/day, tapered in 6 weekly steps to 7.5 mg/day) with sulphasalazine alone in 155 patients. The last assessment of therapy effect of combined treatment was at week 28 of follow-up as prednisolone and methotrexate were tapered and stopped after 28 and 40 weeks, respectively. At week 28, significantly better outcomes in the combined-treatment group were seen in all composite measures and all but one of the core set measures ($P < 0.007$). In these measures, the improvement in the combined-treatment group was 2-fold or almost 2-fold that of the single sulphasalazine group. Seventy-two per cent of the patients in the combined-treatment group *vs* 49% in the sulphasalazine group had improved according to the ACR criteria ($P = 0.006$). The clinical difference between the groups decreased and was no longer significant after prednisolone was stopped, and there were no further changes after methotrexate was stopped. Withdrawal for toxicity during the first 28 weeks was significantly less in the combined-treatment group (1 *vs* 7; $P = 0.04$). The frequency of adverse events not resulting in withdrawal was similar in both groups. In addition to the clinical results, progression of joint damage in the combined-treatment group was one-third that in the sulphasalazine group. This effect persisted until week 80, i.e. 1 yr after the withdrawal of prednisolone which started at week 28 of follow-up.

Van Gestel *et al.* [23] compared the combination of i.m. gold (50 mg/week) and prednisone (initially

10 mg/day for 12 weeks, then tapered to zero in 2-weekly steps) with i.m. gold alone in 40 patients. The main assessment was at week 12, just before prednisone was gradually withdrawn. At this time, all five assessed core set measures showed significantly greater improvement in the combined-treatment group; the magnitude of this improvement is not reported. The improvement in a composite index, the disease activity score (DAS) [39], in the combined-treatment group was more than 2-fold that with single gold treatment. Progression of joint damage was similar in both groups. Withdrawal due to toxicity was the same in both groups after 20 weeks (four patients in each group). The authors report troublesome rebound effects in the combined treatment group after withdrawal of prednisone. This appears to be based on a single significant between-group comparison in an array of 13: in week 20 of follow-up, the DAS in the combined-treatment group was worse than in the control group. However, at this moment (and up to week 44), patients in both groups were still better than at baseline. After 44 weeks (32 weeks after the beginning of tapering prednisone), no between-group difference remained.

Corkill *et al.* [24] compared the addition of three pulses of 120 mg i.m. methylprednisone (at week 0, 4 and 8) to i.m. gold in 59 patients. After 12 weeks, the combined treatment was significantly better in three of four core set measures: improvement in pain and physical function was more than twice as high, and joint count almost twice as high. After 24 weeks, the between-group difference had almost disappeared. Progression of joint damage was similar in both groups. Withdrawal due to toxicity during 24 weeks was more frequent in the combined-treatment group, but not significantly so.

Finally, Ciconelli *et al.* [25] compared the addition of three low-dose i.v. methylprednisone pulses (5 mg/kg), at baseline, month 1 and 2, with sulphasalazine alone in 38 patients. Eighty per cent of the patients in both groups had a prescription of oral corticosteroids. In the 6 month study period, no differences between treatment groups in efficacy or toxicity were found. The relatively low dose of methylprednisone in a population already treated with corticosteroids may have decreased the possible contrast. This important co-intervention with oral corticosteroids was the reason to rate the strength of evidence from this trial as moderate.

Figure 1 summarizes the heterogeneity of the findings of this systematic review graphically. Except for corticosteroids, there appears to be no trend for an overall beneficial effect of a particular drug in a combination. The figure also shows the lack of data: low sample size in most trials, and many untested combinations.

DISCUSSION

In its second update since 1991, this review highlights exciting new developments in the combined drug treatment of RA. In early disease, step-down bridge therapy with corticosteroids appears safe and, in the right dose and duration, truly disease modifying; however, the effect on disease activity (not damage) appears to be dependent on continuation of low-dose corticosteroids. This strengthens Kirwan *et al.*'s [30] finding of the damage control resulting from corticosteroid adjuvant therapy, a study not included in this review because anti-rheumatic therapy was not uniformly applied in the control group. In late disease, patients with a suboptimal response to methotrexate improve clinically with cyclosporin, and some patients on triple therapy with methotrexate, sulphasalazine and hydroxychloroquine appear clinically better off than patients on a two-drug combination or methotrexate alone. This lifts the gloom from the other studies compiled so far, where negative interaction (i.e. results of the combination are the same or only slightly better than the single drugs) prevails, often at the cost of somewhat increased toxicity. Interestingly enough, rheumatologists have not waited for these first positive results or heeded the many negative trials: according to two recent surveys published in abstract [40, 41], they almost universally embrace combination therapy.

Felson *et al.* [42] recently published a meta-analysis on combination therapy in which he pooled the available data. He found a negative answer to the question:

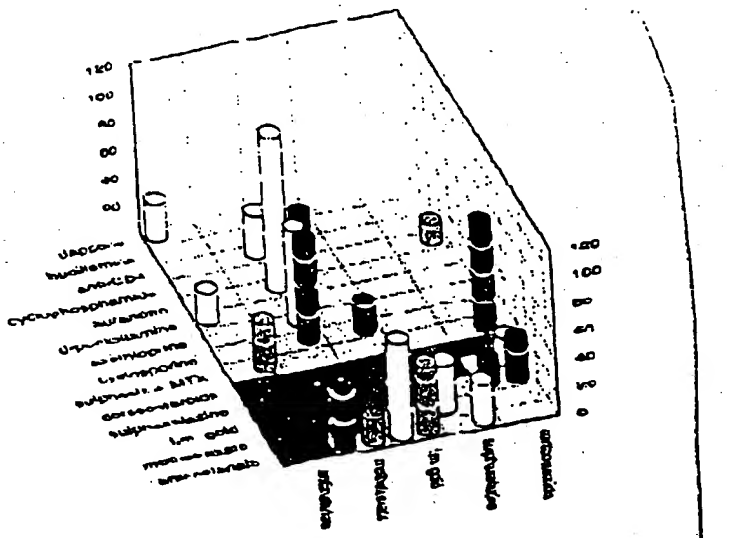

FIG. 1.—Three-dimensional summary of the efficacy of combination therapy. From the perspective of the five most frequently used drugs, the matrix describes the combinations of all single drugs reviewed, and one two-drug combination. Each bar describes a specific combination trial: its length reflects the sample size of the combined-treatment group, its shade reflects the evidence that combined treatment is better than the single drug(s). Dark grey, strong evidence that the combination is better or much better; light grey, moderate evidence that the combination is better (any evidence or trend); white, no evidence that the combination is better. Because the five primary drugs are repeated on the long axis, a dark area in the matrix indicates overlap.

'Does combination therapy on average make a difference compared to average single therapy?' In our view, the heterogeneity in combinations, strategies and patient material makes this a less interesting research question. As shown in Fig. 1, each combination needs careful study of its potential in several trials, which can subsequently be pooled.

Despite the results of Tugwell *et al.*'s study, we feel a step down or parallel strategy in general shows more potential than a step-up strategy. The reason is that step-up trials select patients who have demonstrated less responsiveness to therapy, thus a priori decreasing the chance of future response. Also, if non-compliance is the basis for lack of efficacy, non-compliant patients are more prone to being selected in a trial with non-responders, and the subsequent therapy will again be more prone to fail.

Although methodology has improved significantly in recent years, we still found a number of problems. Most selected studies had small patient numbers, and notably in the studies with negative results, post hoc sample size calculations were often lacking. In theory, type II errors can be minimized by sufficiently large sample sizes. In practice, it is often hard to find eligible patients. Reliable and responsive measurements can also help to record an actual contrast between groups. For example, with joint score assessments, reliability can be improved by frequent training of the assessors.

Co-intervention and contamination are important issues in clinical trials and, obviously, any type of co-intervention should be reported in a transparent way. Co-intervention with low-dose corticosteroids was very common in many of the trials selected for this review. Steroids quickly reduce disease activity. With less room for improvement induced by the investigated treatment, demonstration of contrast between treatment groups becomes harder. Corticosteroids are generally known as symptom-relieving drugs. The data summarized here make it clear that systemic corticosteroids should be considered as disease-controlling anti-rheumatic therapy (DCART) [43]. Accordingly, uncontrolled co-intervention with corticosteroids in RA clinical trials needs reconsideration.

In conclusion, in early RA patients, step-down bridge therapy that includes corticosteroids leads to much enhanced efficacy at acceptable or low toxicity. The effects on joint damage may be persistent, but the symptomatic effects are probably dependent on continued corticosteroid dosing. In late patients, cyclosporin improves a suboptimal clinical response to methotrexate, and the triple combination of methotrexate, sulphasalazine, and hydroxychloroquine appears clinically better than the components. Other combinations are either untested, tested at low sample size, or show negative interaction. In view of the low volume of evidence, most studies need confirmation by replication.

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